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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :		(11) International Publication Number:
C07H 11/00, 11/04, 23/00 A61K 31/70	A1	WO 94/02495
		(43) International Publication Date: 3 February 1994 (03.02.94)

(21) International Application Number: PCT/GB93/01545

(22) International Filing Date: 22 July 1993 (22.07.93)

(30) Priority data:
9216026.6 28 July 1992 (28.07.92) GB

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(81) Designated States: AT, AU, BB, BG, BR, BY, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, US, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: ESTERIFICATION PROCESS

(57) Abstract

An esterification or amidation process comprises reacting a hydroxylated compound or an organic amine with an oxyacid or its anion capable of accepting a lone pair of electrons. The reaction has the advantage that it is carried out under mild conditions in a single step and enables phosphate and other esters of organic compounds (particularly mono- and oligo-saccharides) to be prepared easily.

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ESTERIFICATION PROCESS

The present invention relates to a process for the esterification of hydroxylated compounds.

Esters, particularly phosphate esters, are widespread in nature and occupy a central role in biochemical processes. Of special interest are carbohydrate phosphates which are used extensively for studying biosynthetic pathways, and inositol phosphates which act as secondary cellular messengers. However, because these compounds are often present at low concentration and are usually difficult to isolate in pure form, it is often necessary to synthesise them chemically.

A number of pharmaceutical products possess ester functionalities. Examples include betamethasone, which is used in the treatment of asthma, and menadiol, which is used for haemorrhage control. Both of these compounds are orthophosphates. Phosphate esters are structural features of several bacterial polysaccharides which are used in vaccines to protect against diseases such as pneumonia and meningitis; synthetic antigens with this type of structure have, in recent years, been prepared with a view to using them as synthetic vaccines. It is therefore clear that the introduction of the ester functional group is necessary for the preparation of a number of pharmaceutical products.

Another area in which esters have proved to be useful is in detailed biochemical studies, including studying the effects of structural modifications on the biological activity of various compounds. Such studies include pharmacological testing of new drugs and therapeutic

agents and also investigations into the mechanism of diseases including cancer. In studies such as these, it is often particularly useful to use radioactive esters into which a radioactive isotope of one of the atoms has been incorporated.

It is often impossible to obtain the required ester compounds by any other method than chemical synthesis but, in fact, the chemical synthesis of esters has, in the past, proved to be extremely difficult. Direct methods for the synthesis of esters are available and an example of such a method is the esterification of a hydroxylated compound (HC) using phosphoric acid or polyphosphoric acid (MacDonald, The Carbohydrates, **1A,** 254 (1972) W. Pigman and D. Horton (Eds). Academic Press, New York). However, the utility of this method is limited because many HCs are sensitive to the reaction conditions which are, of course, strongly acidic and often the products undergo further reaction to form modified compounds such as cyclic esters or anhydrides. This route cannot therefore be used for most compounds. Indirect methods of chemical synthesis have also been devised and include those described in the article entitled "Synthesis of Some Low Molecular Carbohydrate Esters of Biological Significance" by Lindh, Chemical Communications, University of Stockholm, (1988), No 8. Further examples are given in the article by Ozaki et al, J. Chem. Soc. Perkin Trans. 1, (1992), 729-737. Both of these articles demonstrate the complexity of known synthetic routes to phosphate esters. Indeed, the latter describes a twelve step synthesis of the product, including several steps which involve chromatographic separations and giving an overall yield of less than 20%.

Clearly, therefore, existing methods for the production of esters are far from ideal and one serious problem is that the extreme reaction conditions necessary for direct esterification lead to unwanted side reactions of both starting materials and products. Attempts have been made to overcome this by using indirect methods of chemical synthesis but these methods usually involve a number of steps selectively to expose specific hydroxyl groups, a number of further steps to introduce the ester and finally a series of deprotection steps. This strategy requires the presence of compatible functional groups in the molecule during the synthesis, which can be limiting. Furthermore, the large number of steps will reduce the overall yield of the final product and often involves the use of large quantities of expensive reagents.

There is therefore a need for a simple and cost effective method of esterifying hydroxyl groups. The present invention is based on the discovery that ester formation can be promoted remarkably easily in solutions containing a hydroxylated compound and an oxyacid salt.

Certain superficially similar processes have previously been applied to starches, for food purposes, as in US-A-2884413, US-A-2865762, US-A-2884412 and US-A-2961440. However, these proposals date from the late 1950s or early 1960s, and the applicability of modified methods for other hydroxylated compounds does not seem to have been appreciated in the intervening 30 to 35 years.

According to a first aspect of the invention, there is provided a process for the esterification of a hydroxylated compound (HC) other than a starch, or the amidation of an organic amine, characterised in that the

process comprises reacting the HC or amine with an oxyacid, oxyacid anion or a mixture thereof wherein the oxyacid or its anion is capable of accepting a lone pair of electrons from the oxygen of the HC or nitrogen of the amine.

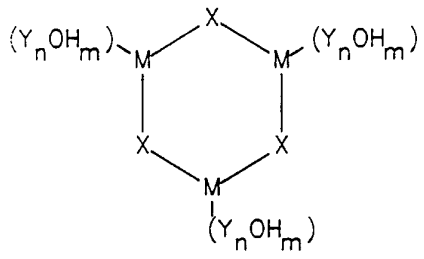
In the context of the present invention, the term "oxyacid" includes within its scope hetero-oxyacids in which one or more of the oxygen atoms have been replaced by a hetero atom, in particular one such as nitrogen, sulphur or a halogen atom. Examples of hetero-oxyacids include thiophosphates, halophosphates and phosphonitriles.

The term "hydroxylated compound" refers to any compound containing a hydroxyl group or an alkoxy or phenoxy anion. Preferred hydroxylated compounds are hydroxylated organic compounds although inorganic compounds, for example oxyacids may be used. Examples of hydroxylated organic compounds include sugars, proteins, glycoproteins, peptides, glycopeptides and glycoconjugates and other molecules having sugar functionality, amino acids, alditols, cyclitols, phosphate esters and carboxylic acids.

The conditions of the process of the present invention are quite surprisingly mild and one particular advantage of this is that, because of the mild pH conditions, it is not necessary to protect (and subsequently to deprotect) other functional groups of the HC. This means that the overall number of steps in the process is reduced and that therefore the overall yield of the final product is increased, thus increasing the cost-effectiveness of the esterification process. Furthermore, the mild reaction

conditions also ensure that the reaction is suitable for nearly any starting material and that there is little likelihood of products undergoing unwanted further reaction.

The oxyacid may be an oxyacid of an element M, which is capable of forming trimeric oxyacids of the structure:



wherein:

M is an electron accepting element;

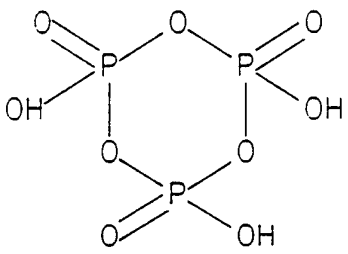
X is oxygen or a hetero-atom such as sulphur or a substituted nitrogen atom;

Y is oxygen, hydrogen or a hetero atom such as sulphur or a substituted nitrogen atom;

n is 0 or 1 and m is 0 to 2, depending on the valency of M.

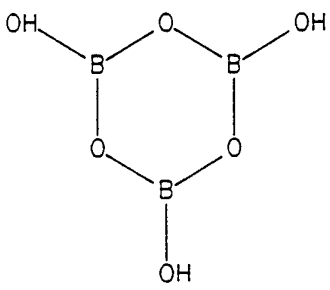
Examples of elements M which are capable of forming trimeric oxyacids include phosphorus, boron and silicon. Usually, the nitrogen atom will be substituted by hydrogen or an alkyl group.

Phosphorus is capable of forming the metaphosphate oxyacid. Because of electron deficiency at the phosphorus atom, the ion tends to polymerise and may form the following trimer:

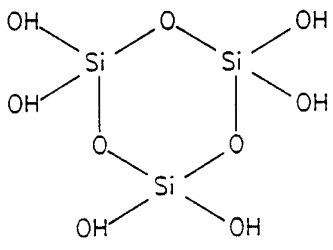


Alternative structures for metaphosphate are cyclic tetramers or chain structures.

Similarly, boron forms an oxyacid having the following structure:



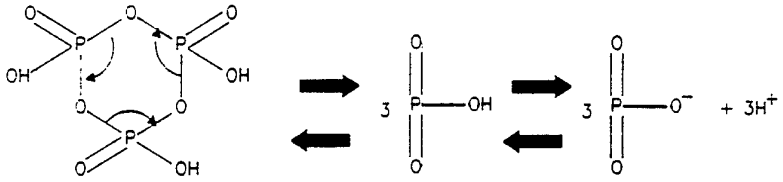
and silicon forms an oxyacid as follows:

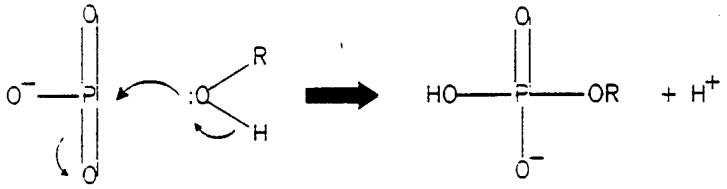


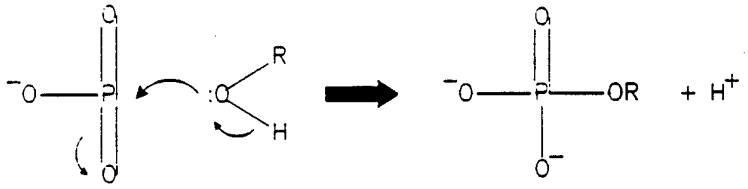
However, the ability of an element to form this type of trimeric structure is merely an indication that one or more of its oxyacids will be capable of accepting a lone

pair of electrons.

One phosphorus oxyacid which has been found to be particularly suitable for use in esterification reactions according to the present invention is metaphosphoric acid and one suggestion for the reaction mechanism for esterification using a metaphosphate is as follows:

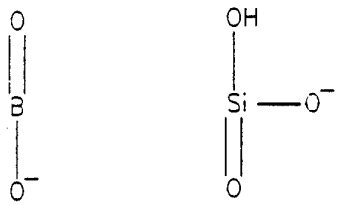






This reaction mechanism indicates that it is the metaphosphate monomer rather than the trimer which is the reactive species. However, the usefulness of the invention is not dependent on the accuracy or otherwise of this reaction mechanism.

The equivalent monomers of the boron and silicon oxyanions are as follows:



and it is likely that they will undergo similar reactions to the phosphate oxyacids and anions.

It is not always necessary to use a salt of the oxyacid anion which forms the type of monomer structure shown above. For example, in the case of phosphorus, although metaphosphate salts are preferred, other oxyacid salts such as orthophosphate, diphosphate, triphosphate, polyphosphate, phosphonate, phosphinate, peroxyphosphate, and hypophosphate salts or their free acids may also be used. If the reactive species for the formation of orthophosphate esters is indeed the metaphosphate monomer, the reactivity of ortho-, di-, tri- and polyphosphate oxyacids can be explained by the fact that in solution these oxyacids and their anions will be in equilibrium with the metaphosphate species *via* which the reaction appears to proceed. Furthermore, when these other phosphates are used, metaphosphate is continuously reacting with the HC and so the equilibrium with the other phosphates will be driven to the metaphosphate side allowing the reaction to continue. An analogous applies to the other species listed above.

Other phosphorus based oxyacids which have been found to be useful in the present invention are hetero-oxyacids such as thiophosphate, halophosphates and phosphonitrilic compounds.

The cation of the oxyacid salt will be such as to ensure that the salt is soluble in the solvent in which the reaction is carried out. In aqueous solution, the cation may be an alkali or alkaline earth metal such as sodium, potassium or magnesium. For certain solvents, particularly organic solvents, larger cations such as lanthanum, caesium or less ionic species such as lithium and ammonium may be more appropriate. In aqueous and in other solvents, the cation may be chosen because of its catalytic effect on the reaction. Preferably, the counter ion will be chosen so that the salt does not have an inappropriate pK value, particularly bearing in mind the preferred pH operating conditions which will be discussed in more detail below.

The reaction can be carried out in both aqueous and organic solvents. When an organic solvent is used it is preferred that it is a highly polar solvent such as dimethyl sulphoxide (DMSO).

In general, the process consists of the following steps:

(a) providing a reaction mixture comprising the HC or amine; an oxyacid or oxyacid salt capable of producing in solution a species capable of accepting a lone pair of electrons from the oxygen of the HC or nitrogen of the amine; and from 0 to 30% (w/w) water based on the weight of the HC or amine;

(b) allowing the HC or amine and the oxyacid species to react to form a product mixture containing an oxyacid ester of the HC or amine; and optionally

(c) either (i) at least partially recovering the ester from the product mixture or (ii) further reacting the ester *in situ* to form a desired compound or mixture.

One method for preparing a suitable reaction mixture is to prepare an aqueous solution of the HC or amine and the oxyacid salt and subsequently to remove water from the solution. Since water is a product of the esterification reaction, it is clear from application of Le Chatelier's principle that the removal of water from the mixture will tend to shift the equilibrium in favour of ester formation and therefore, this method of carrying out the reaction is particularly preferred.

This method of forming the reaction mixture works particularly well if the water in the HC/oxyacid salt solution is initially present in considerable excess (for example, at least 2, 5, 10, 20, 50 or 100 fold (on a weight basis). During the water removal step, most of the water is removed, so as to leave, say, less than one part (for example from 0.1 to 30% w/w), based on the starting amount of HC or amine. As will be recognised by those skilled in the art, a balance has to be struck between considerations of yield, which favour removal of a large amount of water, and considerations of time and energy expenditure, which favours the converse. The optimal place to draw the balance will doubtless vary with the nature of the reactants and the operator's process conditions.

The water can be removed by any appropriate physical or chemical method. Examples of suitable physical methods, which are generally preferred, are evaporation, evaporation under reduced pressure and freeze drying. Considerations of energy consumption and time will often dictate the best in any particular circumstances.

An alternative method for preparing the reaction mixture

is to dissolve the HC or amine and the oxyacid or oxyacid salt in an organic solvent which may also contain water in an amount of up to 30% (w/w) based on the starting amount of HC or amine.

It is important that the oxyacid salt is soluble in the chosen solvent and therefore, a polar solvent such as dimethylsulphoxide should generally be used.

It has been found that in many cases the process is more efficient if the reaction mixture contains water in an amount of from 0.1 to 15% w/w of the HC.

The reaction can be carried out at almost any pH but a preferred pH range is from 1 to 9 and the best results are obtained when the pH is from 2 to 6. However, one advantage of the reaction is that it does work well under mild conditions of pH, for example from pH 4.5 to 6 and this can be particularly important when the HC or the product ester is sensitive to highly acidic conditions. For reactions of amines, the pH will preferably be greater than 7 in order to ensure that a free amine exists in the reaction mixture. Generally, for reaction of amines, the pH will be between 7.5 and 9, although use of pH of 10 or even higher may be necessary in some cases.

Any hydroxylated compound may be esterified or any amine amidated by this method but, in particular, the method is suitable for the esterification of organic compounds such as: sugars, particularly non-reducing sugars such as trehalose and sucrose and reducing-sugars such as lactose and maltose; proteins; glycoproteins, peptides, glycopeptides and glycoconjugates and other molecules

having sugar functionality; amino acids, particularly, serine and threonine; and other organic compounds such as alditols and cyclitols, carboxylic acids, organic oxyacids, particularly phosphates, and compounds containing hydroxyl groups or alkoxy or phenoxy anions. The invention has application to polysaccharides other than starch, oligosaccharides (say with 3 to 10 monosaccharide units) and di- and mono-saccharides.

Inorganic hydroxylated compounds which may be esterified by the method of the invention include oxyacids themselves and, for example, the esterification reaction of the invention is of use for the synthesis of di-, tri- and poly-phosphates.

In general, the reaction is most successful for primary and secondary HCs and amines, although it is possible to esterify tertiary derivatives, particularly if the substituents are not too bulky.

After the formation of the starting mixture, it may then be further treated to maximise the esterification reaction. In some cases, it may be preferable to heat the reaction mixture for a time between 0.1 hour and 40 days at a temperature of from 20 to 200°C, preferably 50 to 100°C. The heating may be carried out in a sealed system, particularly if a high temperature is used and will increase the rate of the esterification reaction. Since water is a product of the esterification process, removal of water from the system as the reaction proceeds will cause the equilibrium to be shifted so that more ester is formed from the reaction. One way of removing the water would be to heat the mixture in an unsealed system allowing water to evaporate.

Once the reaction is complete, the ester or amidate products may be isolated by any suitable method: by ion exchange chromatography, for example. If more than one reaction product is obtained, it is generally easy to separate the different products by chromatography. Alternatively, the ester may be left in the resulting mixture and treated or allowed to react further to produce a compound, or mixture, of choice.

The process of the present invention has made it possible to obtain new esters and amidates which could not be prepared by any previously known route. In particular, it is now possible to prepare esters of acid-labile sugars such as sucrose and trehalose and therefore, in further aspects of the invention, there are provided trehalose-2-phosphate, trehalose-3-phosphate, trehalose-4-phosphate and a number of mono-orthophosphates of sucrose. These compounds are new and could only have been prepared by previously known methods with considerable difficulty and cost.

The invention is further illustrated by the following examples. The examples refer to the accompanying drawings, in which:

FIGURE 1 is a ^1H nmr spectrum of trehalose-6-phosphate obtained from an orthophosphate salt by the method of the present invention;

FIGURE 2 is a ^1H nmr spectrum of trehalose-2-phosphate obtained from an orthophosphate salt by the method of the present invention;

FIGURE 3 is a ^1H nmr spectrum of trehalose-4-phosphate

obtained from an orthophosphate salt by the method of the present invention;

FIGURE 4 is a ^1H nmr spectrum of trehalose-3-phosphate obtained from an orthophosphate salt by the method of the present invention;

FIGURE 5 is a HPLC ion-exchange chromatogram obtained from a sample of α,α'-trehalose treated with sodium phosphate according to the method of the present invention.

FIGURE 6 is a ^1H nmr spectrum of trehalose-3-phosphate obtained from a metaphosphate salt by the method of the present invention.

FIGURE 7 is a ^1H nmr spectrum of trehalose-6-phosphate obtained from a metaphosphate salt by the method of the present invention.

Example 1

The preparation of the four isomeric mono-orthophosphates of α,α'-trehalose.

An aqueous solution of α,α'-trehalose dihydrate (1.0g, 2.6 mmole) in sodium phosphate buffer solution pH 5.5 (0.1M, 100 ml), in which the water is present in an approximately 100 fold excess, on a weight basis, compared to the sugar, is frozen to -78°C (solid CO_2) and is then freeze-dried to give a preparation with a moisture content (determined by Karl-Fischer titration) of 4.5% w/w with respect to trehalose. The preparation, closed to the atmosphere, is heated at 56°C for 10 days

after which it is reconstituted in deionised water (100 ml) and the solution applied to an anion-exchanged column, 120 x 2 cm, Bio-Rad AG 1 resin which had been washed successively with 1M sodium hydroxide, water, 1M sodium acetate, water and is therefore in the acetate form. The expression Bio-Rad AG 1 is a trade mark. The column was first washed with water to remove unreacted trehalose (for recycling if so desired) and any other unbound material, and then eluted with a 0.2 to 0.8M ammonium acetate aqueous solution gradient to give fractions containing the isomeric trehalose mono-orthophosphates separated from inorganic orthophosphate. The fractions containing the trehalose phosphates were then analysed on an analytical ion-exchange column (Dionex BioLC PA 100) and the fractions containing each of the four pure isomers were combined and their solutions freeze-dried to give as analytically pure amorphous solids, trehalose monophosphates as their ammonium salts. The expression Dionex BioLC PA 100 is a trade mark. Fraction 1, 0.085 g (6.6%), fraction 2, 0.035g (2.7%), fraction 3, 0.041g (3.1%), fraction 4, 0.027% (2.1%). Total 0.188g (14.6%). All four products gave, on treatment with the enzyme alkaline phosphates, α,α'-trehalose (identified by HPLC ion-exchange chromatography) and inorganic orthophosphate (identified colorimetrically using ammonium molybdate).

Elemental analysis data:

Fraction 1, found C 31.55%, H 6.62%, N 5.81%, P 6.45%.
Fraction 2, found C 31.22%, H 6.26%, N 5.97%, P 6.39%.
Fraction 3, found C 31.62%, H 6.55%, N 6.20%, P 6.55%.
Fraction 4, found C 31.37%, H 6.32%, N 5.92%, P 6.89%.

$C_{12}H_{29}N_2P_1O_{14}$ requires C 31.58%, H 6.40%, N 6.14%, P 6.79%.

NMR data. The proton nmr spectra of the products present in fractions 1, 2, 3 and 4 are shown in Figures 1 to 4 and are consistent with the 6-, 2-, 4- and the 3-phosphates respectively, i.e structures 1, 2, 3 and 4 shown in the figures. Figure 1 is identical to the proton nmr spectrum of trehalose-6-phosphate - data from Sigma Chemical Co. Confirmation of these structures was also obtained from ^{13}C and ^{31}P nmr spectroscopy.

Example 2

Using conditions similar to those of Example 1, but modified as shown in the six entries (a) to (f) for Example 2 in Table 1, phosphate esters of α,α'-trehalose were prepared from α,α'-trehalose.

Example 3

The eight isomeric mono-orthophosphates of sucrose can be prepared by a similar procedure to that of Example 1, using the conditions shown in Table 1. Of major note in this case, however, is the fact that sucrose is an extremely acid labile disaccharide and consequently is not amenable to direct phosphorylation with phosphoric acid. As with three of the trehalose phosphates above, the majority of the sucrose isomeric esters are new compounds and, furthermore, would be very difficult to prepare using existing methods.

Examples 4 to 7

In an analogous manner

α-glycerophosphate;
serine phosphate;
β-cyclodextrin thiosulphates; and
serine thiophosphate,

were also prepared using the conditions shown in Table 1.

Further experiments were carried out to investigate the effects of sodium phosphate buffer on several HCs. The experiments were carried out in solutions of pH 5.5, 7.0 or 8.1 and all gave, after heating at 56°C for several days, products which co-eluted with carbohydrate monophosphate esters on ion-exchange chromatography. The results of these experiments are shown in Table 1.

A number of experiments have also been carried out to investigate the effects of other oxyacid salt buffers on several HCs at pH 2.0-6.0. After heating at >56°C for several days "ester-type" products are observed on ion-exchange chromatography. The oxyacid buffers which were used include those derived from boric, thiophosphoric, phosphonic and phosphinic acids.

TABLE 1

Example No	Saccharide, protein or amino acid (mg/ml)	solution (molarity)		pH	moisture content* % w/w	degree of esterification*	incubation at 56° Days
2	α,α'-trehalose (10)	sodium phosphate (0.1)	a	5.5	4.8	0.1	2
			b	5.5	4.8	0.3	7
			c	5.5	4.8	1.7	13
			d	5.5	4.8	5.7	38
			e	7.0	11.2	0.05	38
			f	8.1	0.9	0.1	38
3	sucrose (10)	sodium phosphate (0.1)	a	5.5	3.3	2.7	38
			b	7.0	7.3	0.6	38
			c	8.1	0.9	tr	38
4	lactose (10)	sodium phosphate (0.1)	a	5.5	3.3	6.1	38
			b	7.0	8.7	0.1	38
			c	8.1	5.3	tr	38
5	mannitol (10)	sodium phosphate (0.1)	a	5.5	2.1	tr	38
			b	7.0	9.6	tr	38
			c	8.1	13.7	tr	38
6	glycerol (2.6)	sodium phosphate (0.1)		5.5	5.0	0.2	14
7	α,α-trehalose (10)	sodium pyrophosphate (0.1)	a	5.5	11.1	8.7	7
			b	5.5	11.1	12.6	14
		sodium tripolyphosphate (0.1)	a	5.5	11.4	5.2	7
			b	5.5	11.4	6.7	14
8	serine (2.6)	sodium phosphate (0.1)	a	5.5	14.0	1.7	38
			b	7.0	-	0.4	38

Table 1 (contd.)

Example No	Saccharide, protein or amino acid (mg/ml)	solution (molarity)		pH	moisture content* % w/w	degree of esterification*	incubation at 56° Days
9	threonine (3.0)	sodium phosphate (0.1)	a b	5.5 7.0	- -	0.8 0.4	38 38
10	amylose (20)	sodium phosphate (0.1)		5.5	6.0	0.21 (93)	37
11	cytochrome C (10)	sodium phosphate (0.1)	a b	5.5 5.5	1.0 1.0	0.025 (0.1) 0.03 (0.1)	14 22
12	glucose oxidase (7.5)	sodium phosphate (0.1)	a b	5.5 5.5	7.5 7.5	0.07 (3.5) 0.04 (2.3)	14 22
13	bromelain (5)	sodium phosphate (0.1)	a b	5.5 5.5	10.7 10.7	0.10 (1.1) 0.09 (1.0)	12 22
14	bovine serum albumin (20)	sodium phosphate (0.1)		5.5	2.5	0.28 (0.6)	37

*determined by Karl Fischer titration using a Mitsubishi CA100 system. The values are based upon the weight of carbohydrate, amino acid or protein; buffer salts are not included.

*expressed as % by weight of saccharide, amino acid or protein. Assumes an identical detector response factor of oligosaccharides and mannitol to their corresponding phosphates. This is likely to underestimate the amounts of esters present. Glycerol phosphates determined by comparison to α-glycerophosphate. The figures in parenthesis are the calculated average number of phosphorus atoms per molecule of protein or, in the case of amylose, the average number of glucose residues per phosphorus atom.
tr = trace amounts (<0.1%)

It can therefore be seen that the esterification method of the invention is extremely flexible and can be used to esterify a large range of compounds under relatively mild conditions. In addition, the starting materials can be recycled so that increased amounts of product can be obtained.

Example 8

Formation of phosphate esters from preparations of α, α'-trehalose and sodium metaphosphate.

The procedure of Example 1 was repeated except that α,α'-trehalose dihydrate (1.0 g, 2.6 mmol) was dissolved in a solution of sodium metaphosphate (0.1 M, 100 ml). The preparation is frozen to -78° as before, freeze dried, closed to the atmosphere and heated at 56°C for either 5 days or 12 days. The reaction was repeated at different pH values and the results are shown in Table 2 below.

TABLE 2

pH (initial)	% monophosphate 5 days	% monophosphate 12 days
1.9	32	34(7)
3.0	18	23(6)
3.5	15	20(4)
4.0	13	19(4)
4.5	17	15(4)
5.0	13	16(4)
5.5	13	17(4)
6.0	15	12(3)
6.7	11	-
7.2	9	-
8.0	8.5*	-
9.3	3.4*	-
9.9	2.6*	-

* after 7 days

The figures in parentheses are the percentages of diphosphates formed.

The percentage of phosphate shown in Table 2 is determined from detector response and is therefore an underestimate, possibly by a factor as high as 2.

The amount of phosphate formed will depend upon the moisture content and on the temperature at which the reaction is carried out. In several separate experiments carried out at higher temperature but for shorter times, the yield of phosphates increased to about 40% based on

detector response. Again, this is an underestimate and the true value may be as much as twice the detector response value.

Example 9

Reaction of α,α'-trehalose in DMSO.

a) with orthophosphate

α,α'-Trehalose dihydrate (1 part), disodium hydrogen orthophosphate and sodium dihydrogen orthophosphate (4 parts), (the salts mixed in proportions so as to produce a pH of 5.5 when in aqueous solution) were added to dry DMSO (100 parts). The mixture was heated at 80°C for 3 days, and the DMSO was then removed by lyophilisation. The residue was dissolved in water (100 parts) and analysed chromatographically (ion-exchange). The mixture contained about 1% of trehalose phosphates. The relatively low yield is probably due to the poor solubility of the sodium salts in DMSO.

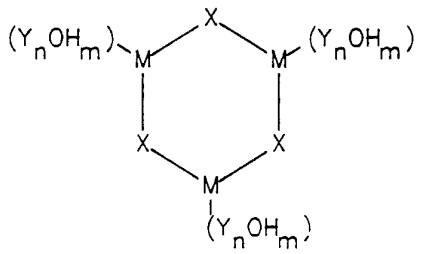
b) with metaphosphate

α-α'-Trehalose dihydrate (1 part), sodium metaphosphate/metaphosphoric acid (4 parts), mixed so as to give a pH of 2 if in aqueous solution, were added to dry DMSO (100 parts) and the mixture heated at 80°C for 3 days. The DMSO was removed and the residue analysed as described above. The mixture contained 1.5% of trehalose phosphates. The relatively low yield is probably due to the poor solubility of the sodium salt in DMSO.

CLAIMS

1. A process for the esterification of a hydroxylated compound (HC) other than a starch, or the amidation of an organic amine, characterised in that the process comprises reacting the HC or amine with an oxyacid, oxyacid anion or a mixture thereof wherein the oxyacid or its anion is capable of accepting a lone pair of electrons from the oxygen of the HC or nitrogen of the amine.

2. A process as claimed in claim 1, wherein the oxyacid is an oxyacid of an element M, which is capable of forming trimeric oxyacids of the structure:



wherein:

M is an electron accepting element;

X is oxygen or a hetero-atom such as sulphur or a substituted nitrogen atom;

Y is oxygen, hydrogen or a hetero atom such as sulphur or a substituted nitrogen atom;

n is 0 or 1 and m is 0 to 2, depending on the valency of M.

3. A process as claimed in claim 1 or claim 2, wherein M is phosphorous, boron or silicon.

4. A process as claimed in any one of claims 1 to 3, wherein the oxyacid species is derived from a metaphosphate, orthophosphate, diphosphate, triphosphate, polyphosphate, phosphonate, phosphinate, peroxyphosphate, hypophosphate, thiophosphate, halophosphate, phosphonitrilic, borate or silicate salt or the free acid thereof.

5. A process as claimed in any one of claims 1 to 4, comprising:

(a) providing a reaction mixture comprising the HC or amine; an oxyacid or oxyacid salt capable of producing in solution a species capable of accepting a lone pair of electrons from the oxygen of the HC or nitrogen of the amine; and from 0 to 30% (w/w) water based on the weight of the HC or amine;

(b) allowing the HC or amine and the oxyacid species to react to form a product mixture containing an oxyacid ester of the HC or amine; and optionally

(c) either (i) at least partially recovering the ester from the product mixture or (ii) further reacting the ester *in situ* to form a desired compound or mixture.

6. A process as claimed in claim 5, wherein the reaction mixture is prepared by forming an aqueous solution of the HC or amine and the oxyacid salt and removing water from the solution to form the starting mixture.

7. A process as claimed in claim 6, wherein the water

is initially present in at least 2 fold excess, on a weight basis, compared to the starting amount of HC or amine.

8. A process as claimed in claim 6 or claim 7, wherein the water is removed by evaporation, evaporation under reduced pressure or freeze-drying.

9. A process as claimed in claim 5, wherein the reaction mixture is prepared by dissolving the HC or amine and the oxyacid salt in an organic solvent, wherein the organic solvent contains water in an amount of from 0 to 30% (w/w) based on the amount of HC or amine.

10. A process as claimed in claim 9, wherein the solvent is dimethyl sulphoxide.

11. A process as claimed in any one of claims 1 to 10, wherein the amount of water in the reaction mixture is not greater than 15% (w/w) based on the weight of the HC.

12. A process as claimed in any one of claims 1 to 11, wherein the pH of the mixture is from 1 to 9.

13. A process as claimed in claim 12 wherein the pH of the mixture is from 2 to 6.

14. A process as claimed in any one of the preceding claims wherein the HC comprises a sugar, a protein, a glycoprotein, a peptide, a glycopeptide, a glycoconjugate, an amino acid, an alditol or a cyclitol.

15. A process as claimed in claim 14, wherein the sugar is a reducing sugar.

16. A process as claimed in claim 14, wherein the sugar is a non-reducing sugar.

17. A process as claimed in claim 14, wherein the amino acid is serine or threonine.

18. A process as claimed in any one of claims 1 to 17, including the step of heating the mixture for from 0.1 hours to 40 days at a temperature from 20° to 200°C.

19. A process as claimed in claim 18, wherein the temperature is from 50° to 100°C.

20. A product mixture obtainable by a process as claimed in any one of claims 5 to 19.

21. A reaction mixture obtainable by a process as claimed in any one of claims 5 to 19.

21. A reaction mixture containing a hydroxylated compound or an organic amine, an oxyacid or oxyacid salt capable of producing, in solution, an oxyacid species which is capable of accepting a lone pair of electrons and water, wherein the water is present in an amount of from 0.1 to 30% (w/w) based on the weight of the HC or amine.

22. Trehalose-2-phosphate;
trehalose-4-phosphate;
trehalose-3-phosphate; or
a mono-orthophosphate of sucrose.

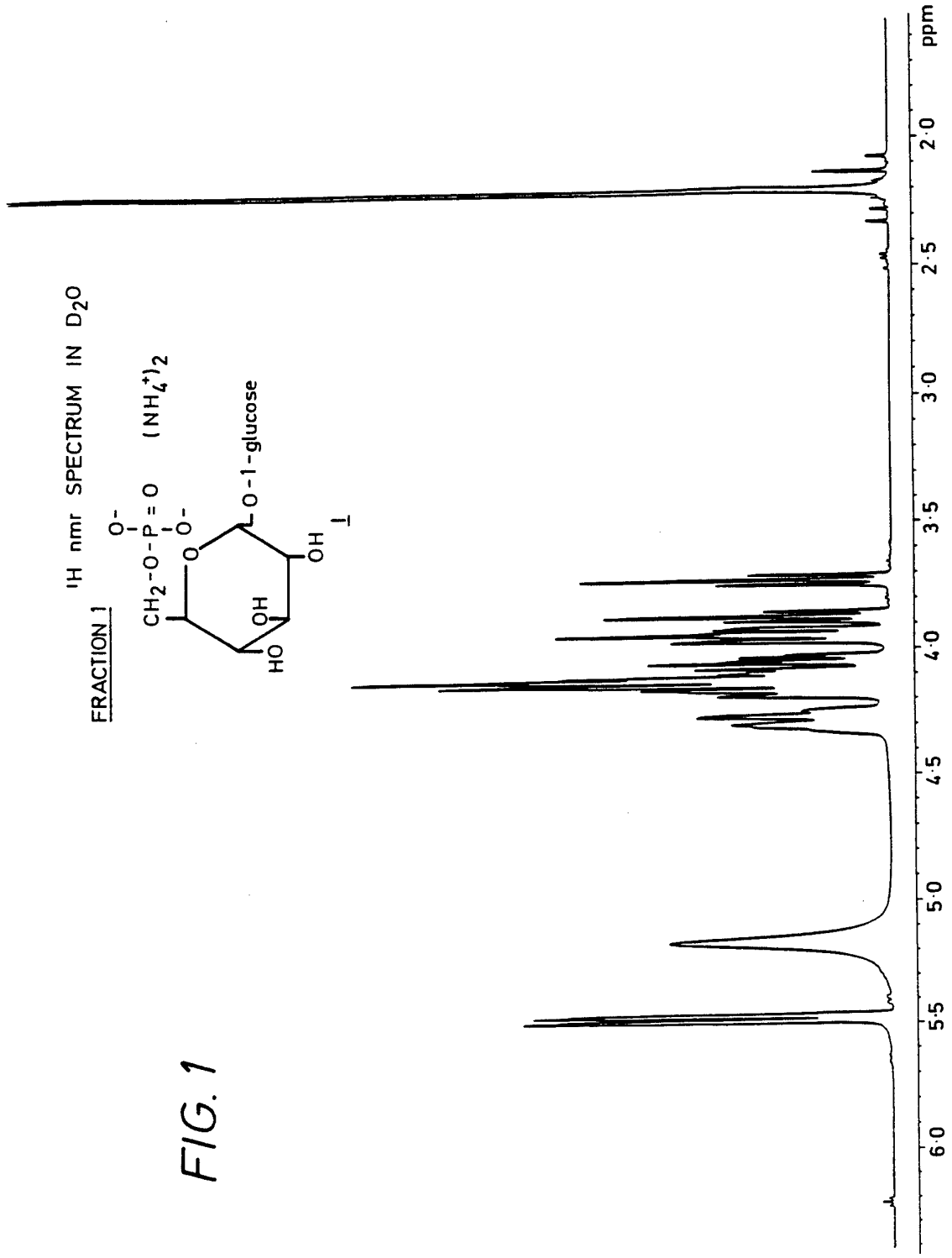

FRACTION 1
^{1}H nmr SPECTRUM IN D_2O
$(NH_4^+)_2$
O-1-glucose
ppm
6·0
5·5
5·0
4·5
4·0
3·5
3·0
2·5
2·0

FIG. 1

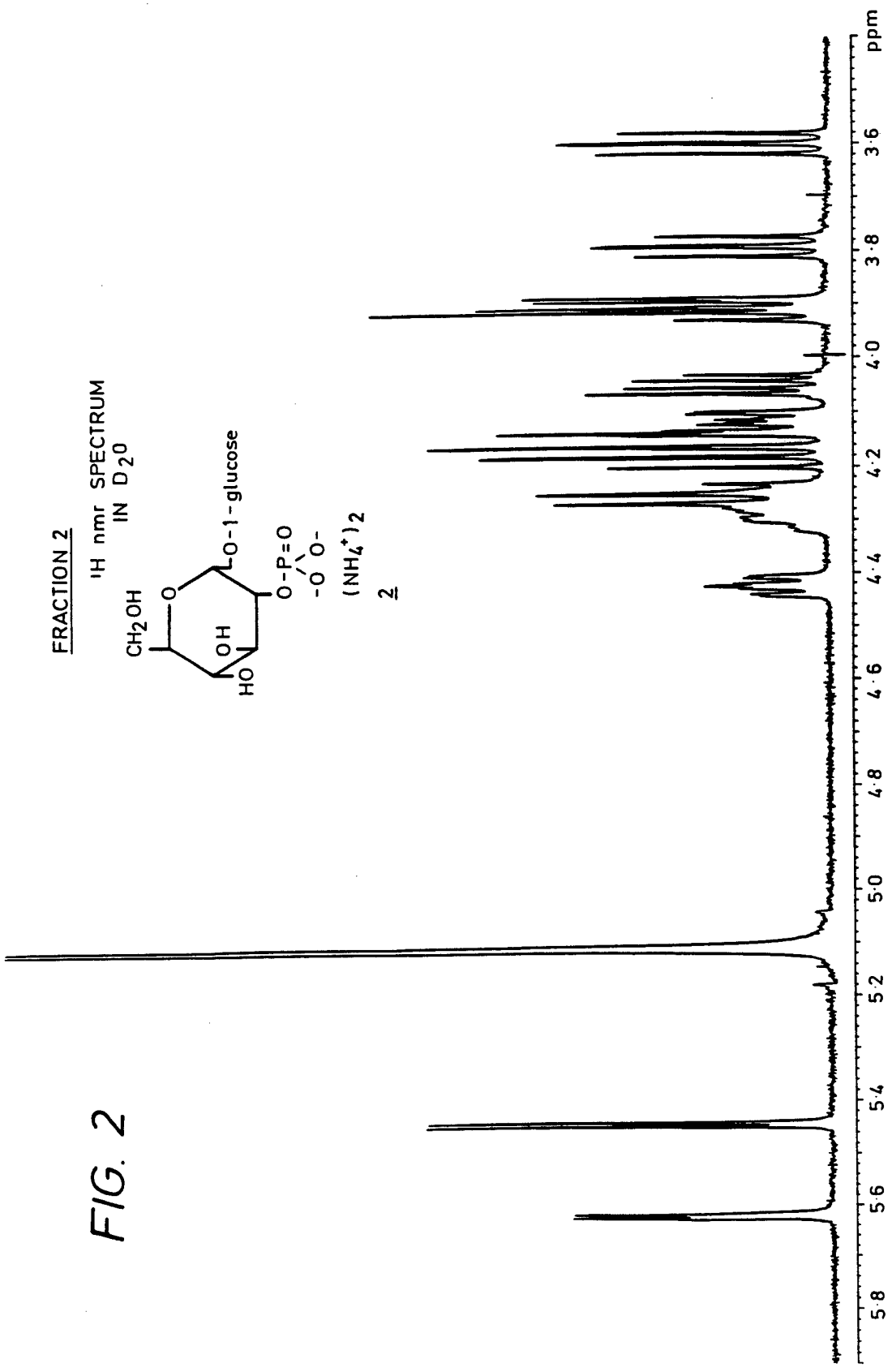

FRACTION 2
^{1}H nmr SPECTRUM
IN D_2O
CH_2OH
O
OH
HO
O-1-glucose
O-P=O
-O O-
$(NH_4^+)_2$
2
5·8
5·6
5·4
5·2
5·0
4·8
4·6
4·4
4·2
4·0
3·8
3·6
ppm

FIG. 2

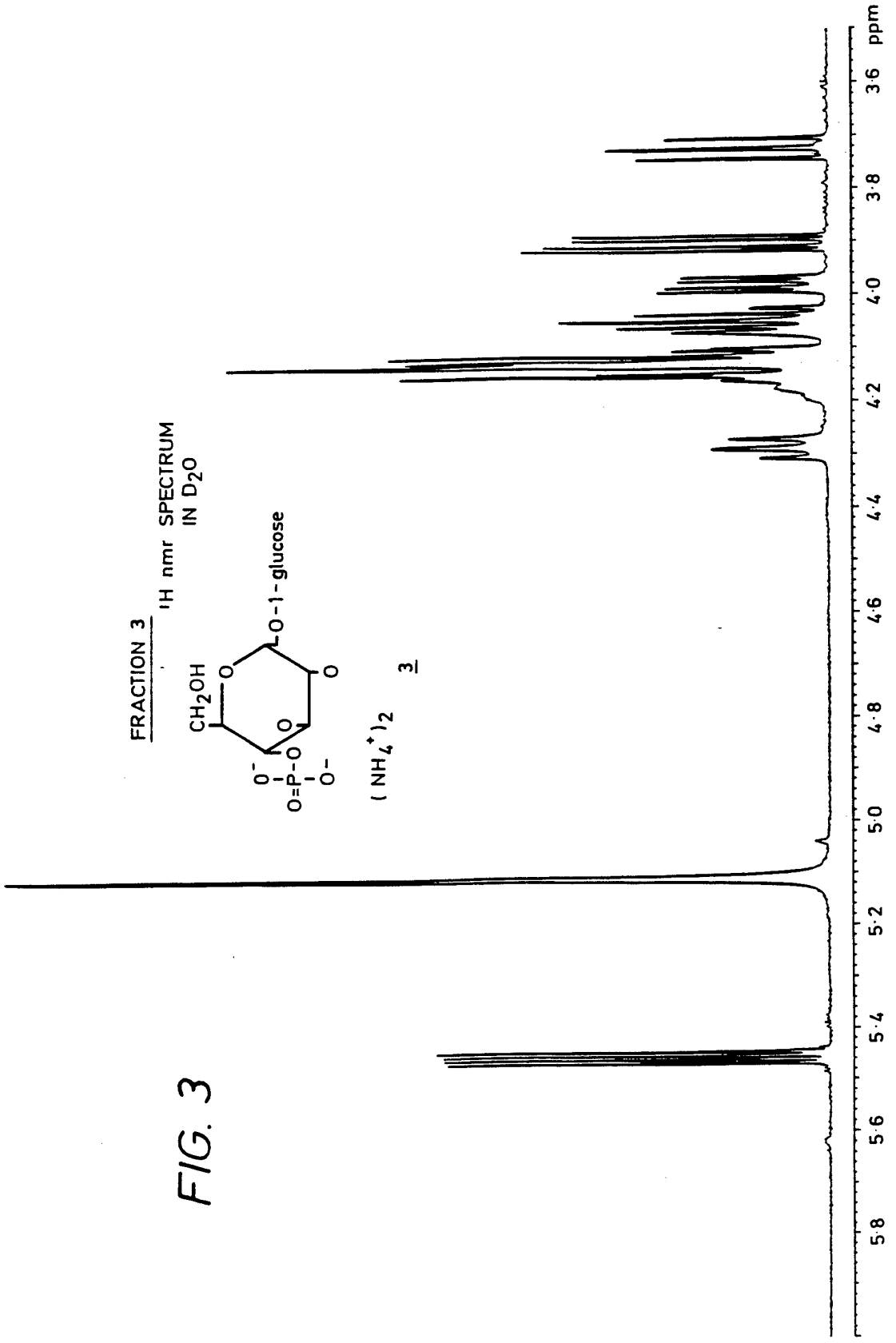

FRACTION 3
^1H nmr SPECTRUM
IN D_2O
CH_2OH
O-1-glucose
$O=P-O$
$(NH_4^+)_2$
3
5·8
5·6
5·4
5·2
5·0
4·8
4·6
4·4
4·2
4·0
3·8
3·6
ppm

FIG. 3

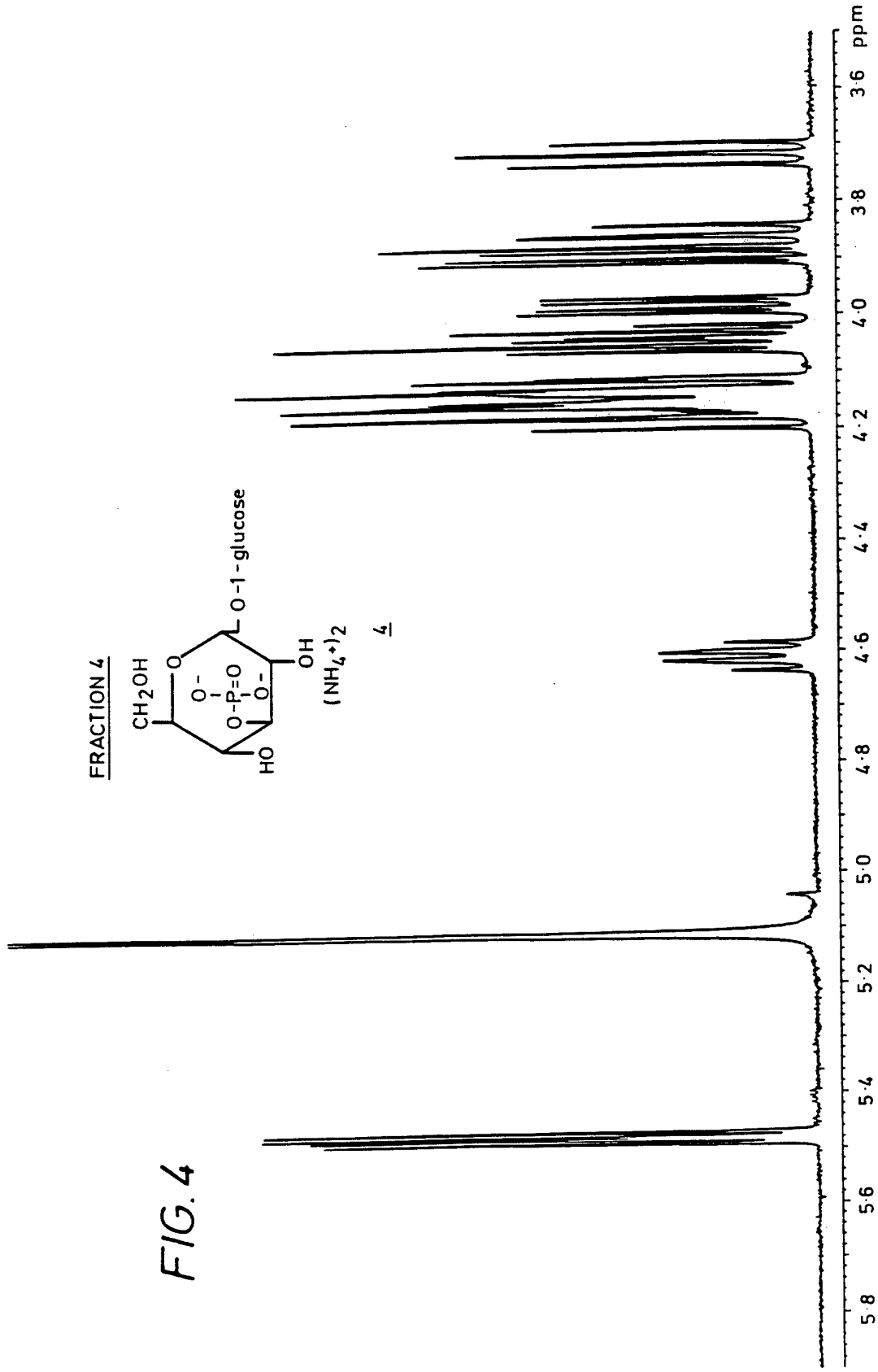
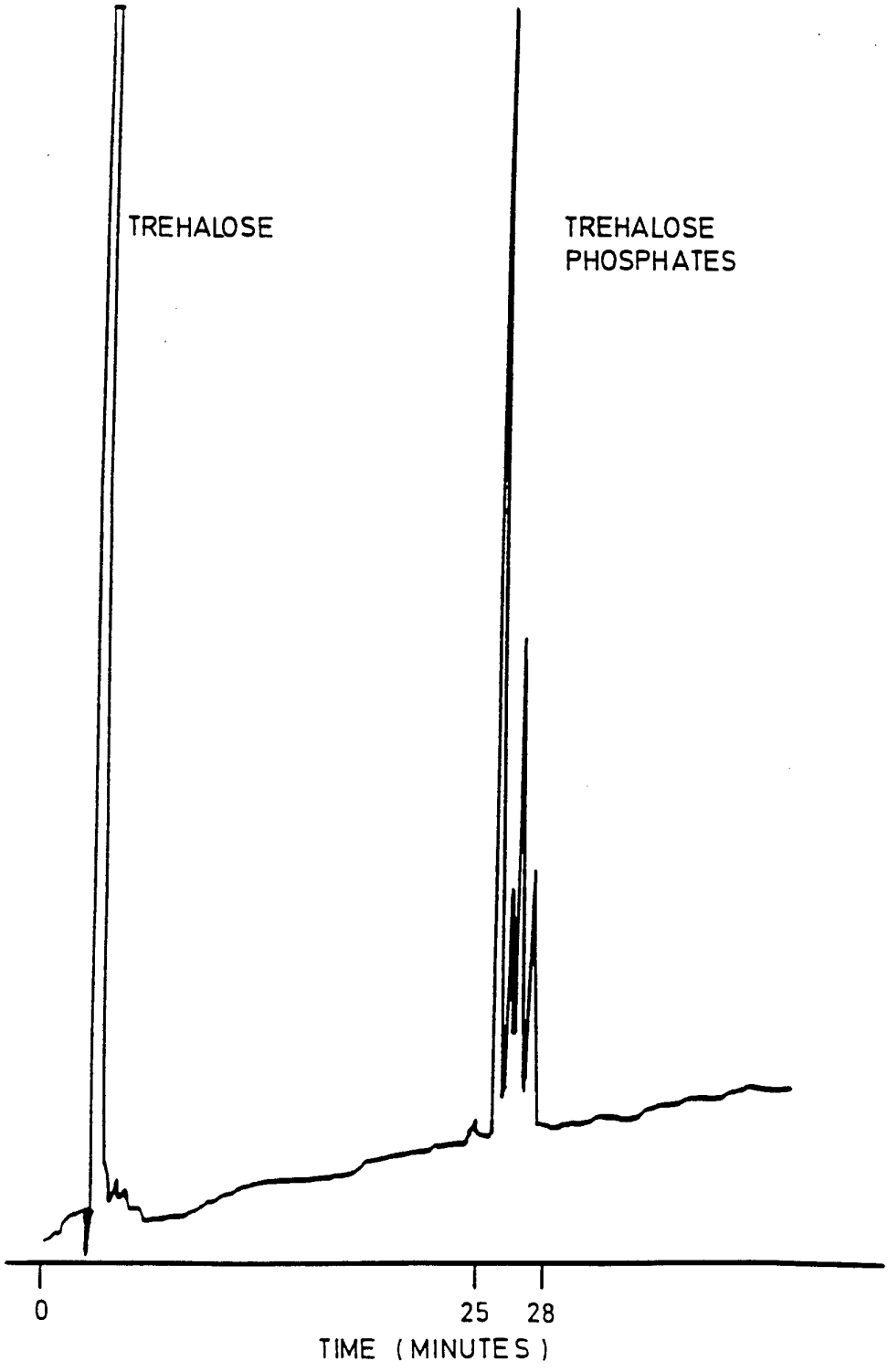

FRACTION 4
CH2OH
O
O-1-glucose
O-
O-P=O
O-
HO
OH
(NH4+)2
4
5·8
5·6
5·4
5·2
5·0
4·8
4·6
4·4
4·2
4·0
3·8
3·6 ppm

FIG. 4

FIG. 5



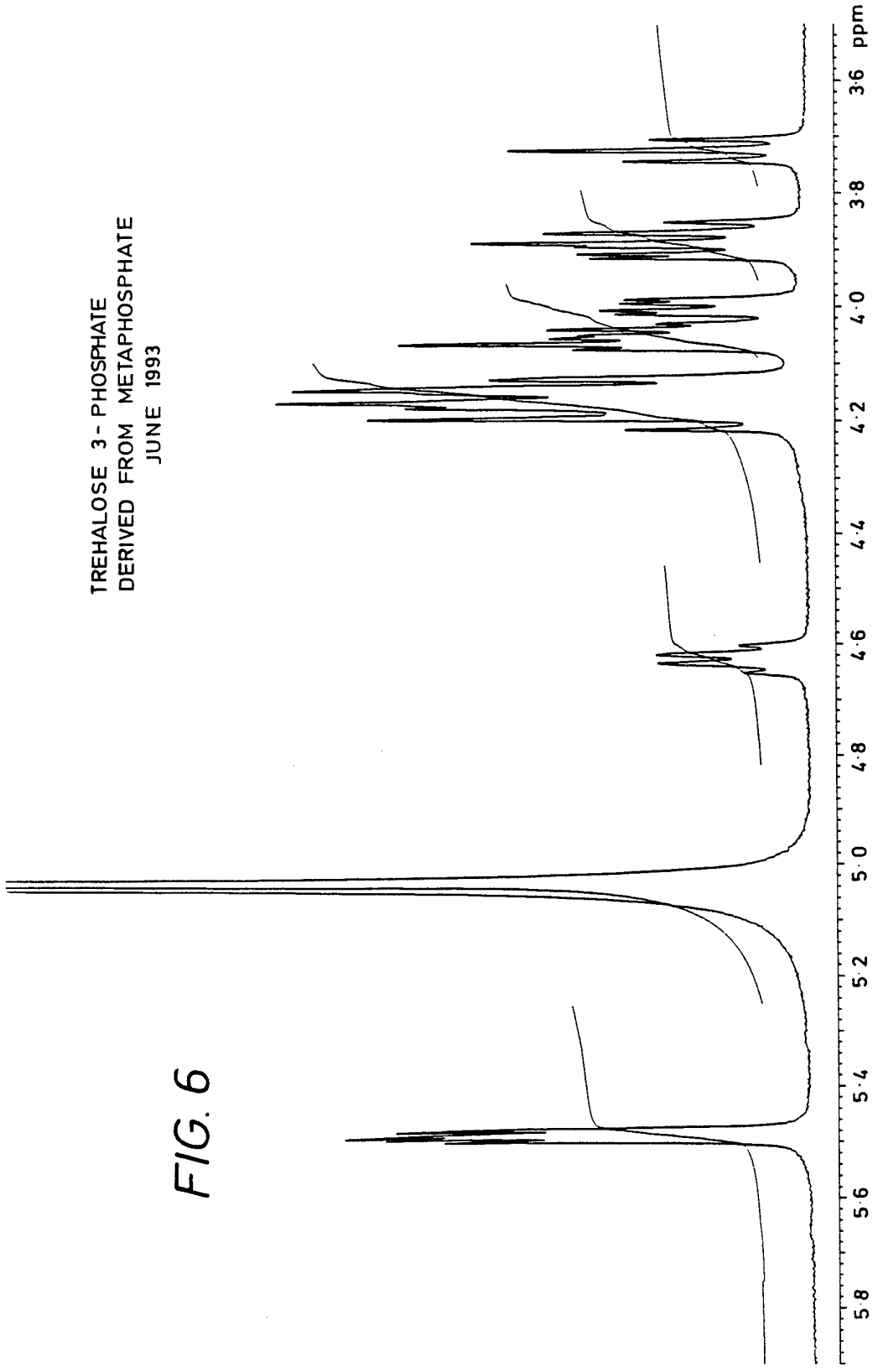

TREHALOSE 3 - PHOSPHATE
DERIVED FROM METAPHOSPHATE
JUNE 1993
5·8
5·6
5·4
5·2
5·0
4·8
4·6
4·4
4·2
4·0
3·8
3·6 ppm

FIG. 6

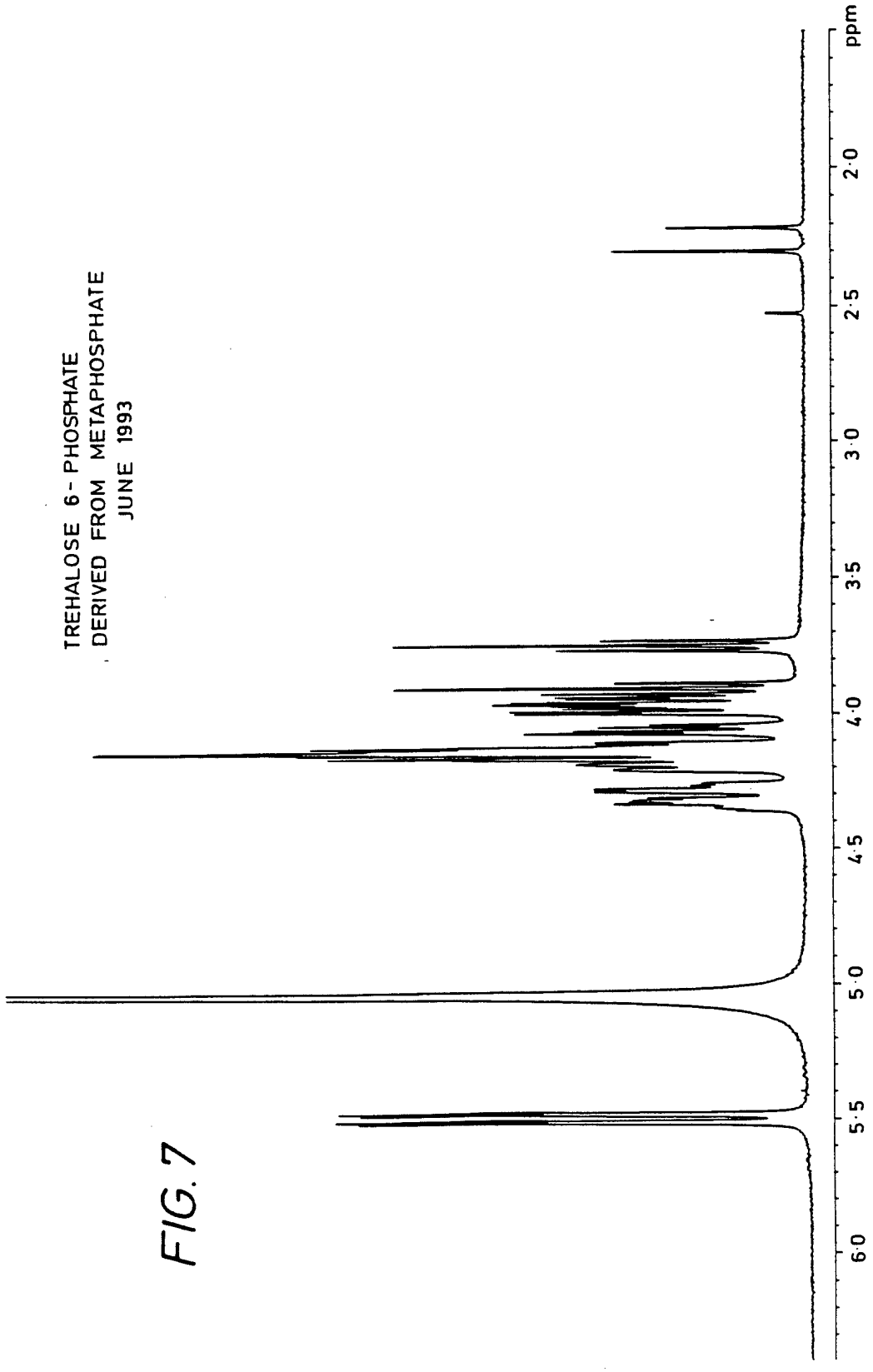

TREHALOSE 6- PHOSPHATE
DERIVED FROM METAPHOSPHATE
JUNE 1993
ppm
6·0
5·5
5·0
4·5
4·0
3·5
3·0
2·5
2·0

FIG. 7

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 93/01545

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: C 07 H 11/00,C 07 H 11/04,C 07 H 23/00,A 61 K 31/70

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]	C 07 H,A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	EP, A2, 0 228 612 (SS PHARMACEUTICAL CO., LTD.) 15 July 1987 (15.07.87), claims. --	1,22
A	US, A, 2 961 440 (R.W. KERR et al.) 22 November 1960 (22.11.60), claims. --	1
A	US, A, 2 884 413 (R.W. KERR et al.) 28 April 1959 (28.04.59), claims. --	1
A	US, A, 2 884 412 (H. NEUKOM) 28 April 1959 (28.04.59), claims. --	1
A	US, A, 2 865 762 (H. NEUKOM) 23 December 1958	1

* Special categories of cited documents: [14]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
29 September 1993	03. 11. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	SCHNASS e.h.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages	Relevant to Claim No.
	(23.12.58), claims 1,2. ----	

ANHANG

zum internationalen Recherchenbericht über die internationale Patentanmeldung Nr.

ANNEX

to the International Search Report to the International Patent Application No.

ANNEXE

au rapport de recherche international relatif á la demande de brevet international n°

PCT/GB 93/01545 SAE 77026

In diesem Anhang sind die Mitglieder der Patentfamilien der im obengenannten internationalen Recherchenbericht angeführten Patentdokumente angegeben. Diese Angaben dienen nur zur Unterrichtung und erfolgen ohne Gewähr.

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The Office is in no way liable for these particulars which are given merely for the purpose of information.

La présente annexe indique les membres de la famille de brevets relatifs aux documents de brevets cités dans le rapport de recherche international visée ci-dessus. Les reseignements fournis sont donnés á titre indicatif et n'engagent pas la responsibilité de l'Office.

Im Recherchenbericht angeführtes Patentdokument Patent document cited in search report Document de brevet cité dans le rapport de recherche	Datum der Veröffentlichung Publication date Date de publication	Mitglied(er) der Patentfamilie Patent family member(s) Membre(s) de la famille de brevets	Datum der Veröffentlichung Publication date Date de publication
EP A2 228612	15-07-87	CA A1 1263649	05-12-89
		DE C0 3674752	08-11-90
		EP A3 228612	13-01-88
		EP B1 228612	03-10-90
		JP A2 62174094	30-07-87
		JP B4 5029235	28-04-93
		US A 4814436	21-03-89
US A 2961440		keine - none - rien	
US A 2884413		keine - none - rien	
US A 2884412		keine - none - rien	
US A 2865762		keine - none - rien	